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An Experiment in Diffusion, Water Pollution, and Bioassay Using Polyethylene Film as A Semipermeable Membrane

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A novel experiment in aquatic biology employing polyethylene bags can be utilized by large groups. The author is Professor of Biology at Winona.

Introduction

Water-filled polyethylene bags are permeable to oxygen. They thus provide excellent devices for measuring the oxygen concentration of waters which are so full of pollutants or particulate matter that they are virtually impossible to test by conventional means (2). A water-filled bag is placed in the water to be tested and it is allowed to remain there for at least 24 hours. Oxygen diffuses through the polyethylene membrane until the concentration of dissolved oxygen in the water within the bag is equal to the concentration of oxygen in the water surrounding the bag. The water in the bag is then tested by standard methods for its dissolved oxygen

concentration (1, 3).

A small fish or other aquatic organism can be placed in another waterfilled bag. By its living or dying in the test situation, the bioassay organism is also used as a measure of the dissolved oxygen concentration of the surrounding medium.

I have found that modifications of these methods, which were developed for research work, provide a novel approach whereby students may learn, by experimentation, about water pollution, bioassay, and about the diffusion of a gas through a semipermeable membrane. The method is so flexible that its applications are limited only by the imagination of the instructor.

The experiment is easily adapted to large

classes which have many sections. I use this experiment in a class of 120 students (5 lab sections), yet each student is an active participant—not merely an observer.

Objectives

The objectives of the experiment are; (1) to determine if oxygen will diffuse through a polyethylene membrane; (2) to determine how a high biochemical-oxygen demand pollutant such as milk will affect the dissolved-oxygen content of water; (3) to determine how apparently innocuous substances such as milk can kill aquatic organisms.

Materials Needed

The following materials are needed to perform the experiment: polyethylene bags (8 in x 15 in, 0.05 mm thick), aquarium aerator, dissolved-oxygen testing kit, hardy bait minnows such as fat heads or blunt-nose minnows, heavy string, 3 x 5 cards to make labels, a supply of tempered (dechlorinated) water, large containers such as tubs, garbage cans or aquaria and milk. The standard Winkler titration method of oxygen determination can be used, but I have found that a simple colorimetric modification of the test is best for this experiment (the Hach Chemical Company of Ames, Iowa,

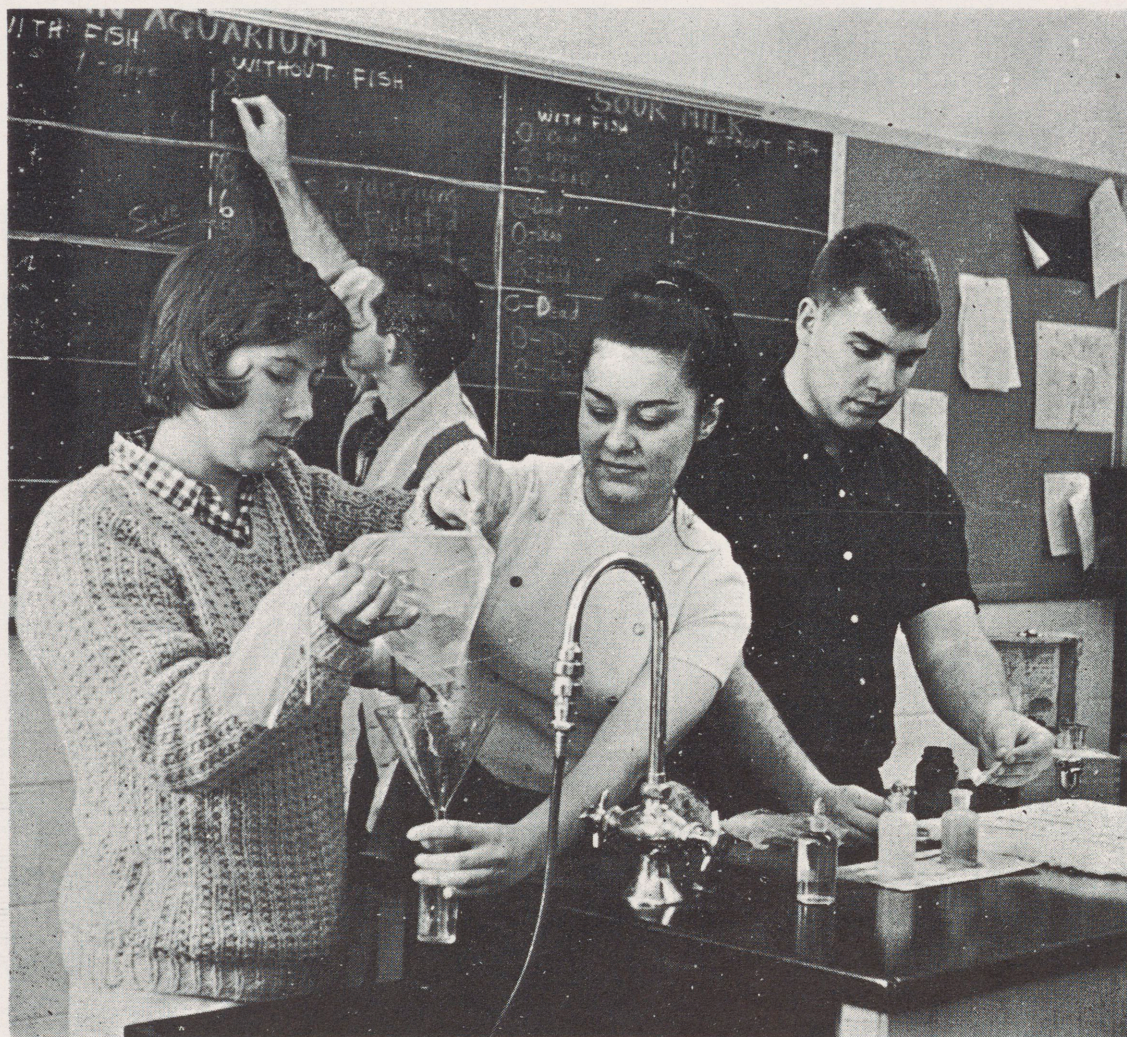


Fig. 1. Students determine the dissolved-oxygen concentrations of the water in experimental aquaria and tabulate their results on the board.

sells a very handy, inexpensive, colorimetric dissolved-oxygen testing kit). Tap water which has been allowed to stand for three days may be considered to be dechlorinated. If you are in a hurry, use hot tap water, let it cool over night and aerate it prior to use.

Procedure

The members of the class should work in pairs. Each pair will be a member of a group. There will be three groups. Determine, at this time, the dissolved-oxygen concentration of the water which is to be used to fill the bags. The simple directions for this procedure are provided with the kit.

Group I. One member of each pair in Group I should place a minnow in a bag which has been filled with two liters of dechlorinated water. Be sure that the water in the minnow bucket and the water in the bag are the same temperature, otherwise the sudden change of temperature may kill

the minnow. The student's name and group number are written on a label in *pencil*. The label is placed *in* the bag so that it will not become lost later. Squeeze the air out of the neck of the bag and tie the neck of the bag securely with string. It will take two people to do this. Try not to have any air bubbles in the bag. Check the bag for leaks. If the bag leaks, discard it. The other member of the pair prepares a bag in exactly the same manner, but he leaves the minnow out. All members of Group I should leave their bags on a laboratory table where they will not be disturbed. Group I will thus determine if oxygen will diffuse from air through the polyethylene membrane into the water, thus enabling the fish to live. The bags should be left in place for at least 48 hours.

Group II. Use the same procedure as Group I except that all of the bags are



Fig. 2. The polyethylene bag full of test water is held over a funnel and the tip of the bag is cut off.



Fig. 3. The bag is quickly lowered into the funnel. The weight of the water in the bag makes an airtight seal against the funnel. The test water is thus transferred, without aeration, into the dissolved-oxygen testing bottle.

placed in a large tank which contains tempered water. Group II serves as a control for experimental Group III. If over 20% of the volume of the tank is filled with water-filled bags, an aerator may be necessary. Allow five minnows to swim free in the tank.

Group III. Use the same procedure as Group II except that the bags will be placed in an identical container of water to which milk has been added (about one quart of milk per 20 gallons of water). The bacterial respiration concerned with the digestion of the milk will deplete the dissolved-oxygen supply of the water. If the room is very cool, however, or if the water still contains chlorine the process will proceed slowly. Best results are obtained if the milk is slightly soured at the outset. The bags should be completely immersed in the milky water lest oxygen diffuse in through an exposed portion of a bag. Five fish should be allowed to swim free in the milky water.

After 48 hours or more the bags from all three groups will be examined. The bags which contain fish will be examined to determine whether the fish lived or died. The minnows which swam free will also be checked. The water in all bags will then be tested for dissolved-oxygen concentration. All data will be recorded in tabular form on the blackboard (Fig. 1). Each student will record the data from all laboratory sections into his notebook.

The oxygen determination is made in the following manner. Immediately after being removed from its test location, the bag is raised and held over a large funnel which rests in a standard dissolved-oxygen sample bottle (Fig. 2.) The stem of the funnel has previously been extended to the bottom of the bottle with rubber tubing. It is important at this point to not stand in line with bag in hand because oxygen diffuses very rapidly into the bag from the air. The bag is held over the funnel, the extreme tip of the lowest corner is cut off with scissors (Fig. 3), and the bag is quickly lowered into the funnel. The weight of the water in the bag will make an airtight seal between the bag and the sides of the funnel. The first water to enter the bottle is undoubtedly oxygenated, but the quantity of water in the bag is sufficient to flush the

bottle adequately. The sample is then treated for its dissolved-oxygen concentration according to the standard Winkler method. I prefer the simplified colorimetric version of this test for beginning students because they do not lose sight of the original objectives of the experiment as they do when they become involved in titration procedures. Even the slowest student quickly associates a white milky color with a low level of dissolved oxygen. It is equally easy for him to associate a bright orange color with a high dissolved-oxygen concentration. Advanced students may wish to titrate, however, to obtain more exact determinations (1, 3). Advanced students may also be referred to the theoretical considerations presented in an earlier paper (2).

Discussion

The students will be quick to notice, as they observe all data recorded in tabular form on the blackboard, that milk is a pollutant. Somehow, it decreased the dissolved oxygen content of the water and killed fish. Each student should make a list of conclusions based on the experiment. The following are typical of some of the conclusions that students have drawn from this experiment; (1) polyethylene film is permeable to oxygen; (2) polyethylene film is not permeable to water; (3) polyethylene film is, therefore, a semipermeable membrane; (4) milk, which is non-poisonous when taken internally, is a high biochemical oxygen demand (B.O.D.) pollutant and as the milk undergoes bacterial decomposition it can kill sensitive aquatic species by depleting their oxygen supply; (5) conditions of oxygen depletion can be detected by using a fish as a bioassay organism; (6) oxygen diffuses faster from the air through a polyethylene membrane into water than it does from water through the membrane into water.

Equally important, the instructor should point out conclusions which *cannot* be drawn from the experiment. The following conclusions are typical of those which *cannot* be drawn from this experiment. Polyethylene film is permeable to carbon dioxide (although this is true, we did not test for it). All fish need at least 4 p.p.m. of oxygen to

survive at room temperature (we can only be sure of the requirements of one species—the one which we used in the experiment).

Suggestions for Other Experiments

The applications of this general method seem endless. The following are some modifications which I have used; (1) put different numbers of minnows in each bag to determine how many minnows it takes to exceed the diffusion rate of the membrane; (2) use 10 bags with a fish in each. Excite half of the fish so that they swim constantly. Allow the others to remain undisturbed. Check the dissolved oxygen content of the water in all bags to determine what effect increased respiration has had; (3) use small crayfish or other aquatic organisms as bio-assay organisms; (5) use sugar or hamburger or too much goldfish food as a pollutant instead of milk (Don't we often kill aquarium fish by polluting the water with excess food?); (6) vary the temperature; (7) put 12 bags of water in soured milk and remove

them all into the air after two days. The dissolved-oxygen concentration within the bags should now be very near zero. Check the dissolved-oxygen concentration of the water in one of the bags every two hours. Plot a diffusion-rate curve. The students should find that the rate of oxygen diffusion is a logarithmic function and that when equilibrium is approached the curve becomes asymptotic. Clever students will quickly devise a valid method by which no one has to stay up late at night to run the analyses.

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